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BY: Carla Wight DATE: November 7, 2003

PATENT
MAIL STOP RCE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of Henry, <i>et al.</i>	: Group Art Unit 1638 : : :
Appln. No:	09/839,813	: Examiner: S. Baum : :
Filed:	April 20, 2001	: Attorney Docket No. : :
Title:	NEW PLANTS FORMED BY MICROPARTICLE BOMBARDMENT WITH UNCHARACTERIZED DNA	: 054195-5001 : :

DECLARATION OF LEONARD SLADE LEE PURSUANT TO 37 C.F.R § 1.132

I hereby declare as follows:

1. I, LEONARD SLADE LEE of 100 Lindendale Rd, Lismore, New South Wales, 2480, Australia, have read the above-referenced United States Patent Application and consider myself to be experienced in the art of plant genetic engineering to which the above-mentioned United States Patent Application pertains. I attach herewith a copy of my *Curriculum Vitae*, illustrating that I am currently Associate Professor of Research (Molecular Genetics) at Southern Cross University. I obtained a PhD from University of Wollongong, New South Wales, Australia, in 1995 and was appointed Associate Professor at Southern Cross University in 1997.

2. I am aware of the Examiner's reasons for rejecting Claims 22-25, 27, 28 and 30-31 of the instant application as obvious over Turbin *et al.*, (1975) in view of Christou (1997) as outlined in the Office Actions dated November 21, 2002, and July 9, 2003. The Examiner has asserted that "*It would have been within the scope of one of ordinary skill in the art to modify the method of Turbin et al. and to use the microparticle bombardment method as taught by Christou*". Allegedly, the motivation is provided by Christou who states that

microprojectile bombardment is “the best method for achieving truly *genotype-dependent transformation*”.

3. In my opinion, Turbin *et al.*, (1975) is a document that provides data that is considered dubious and unreproducible and has been largely ignored by persons of ordinary skill in plant genetic engineering. This is not merely my conclusion but is also that of other authors in this field. In light of this, it is my opinion that there would have been no motivation to combine Turbin *et al.*, 1975, *supra* with Christou (1997) at the time of filing of the present application, in the manner suggested by the Examiner. More particularly, if indeed “*microprojectile bombardment is the best method for achieving truly genotype-dependent transformation*” as asserted by the Examiner in his reading of Christou (1997), then with this in mind, a person of ordinary skill in the art would not be motivated to modify the dubious method of Turbin *et al.*, (1975) by adopting the teaching of Christou (1997).

4. My reasoning underlying this conclusion is set out in the following paragraphs and relies, at least in part, on papers published by others in the field of plant transformation technology as referenced in this present Declaration.

5. Turbin *et al.*, (1975) is cited only ten times in the entire English language scientific literature since 1975 (and one report in Russian); three of these citations were publications by one or more of the authors of Turbin *et al.*, (1975) and provide only cursory allusions to this paper. It is generally apparent that the method of Turbin *et al.* (1975) has attracted little acclaim and has not been substantially reproduced. The most recent of these ten citations was nearly 20 years ago (1984).

6. The remarks of Kleinhofs & Behki (1977) threw considerable doubt on the rigour of the Turbin *et al.* (1975) methods and conclusions, in regard to efficient transformation. Kleinhofs and Behki point out at page 92 that, *these experiments indicate that only “slightly deproteinized highly polymeric DNA from barley endosperm material was successful in bringing about this transformation. Actually only one treated plant, No. 506/19, produced wild-type pollen grains in a high frequency. No controls with similarly prepared DNA from wx plants were reported”*.

7. Furthermore, Kleinhofs & Behki (1977) cast doubt upon a subsequent analysis of the progeny of 506/19 which had shown high correlation between wild-type pollen and two-rowed barley (DNA donor) on the one hand, and waxy pollen grains and six-rowed barley (recipient), on the other. They go on to say that, *"The correlation between waxy and six-row types, surprising, since the genes are located on different chromosomes, was not explained."*

8. Five years after the publication of Turbin *et al.*, 1975, *supra*, in a detailed review of the state of the technology, Kado & Kleinhofs (1980) - assessing more than 200 references - make only a single allusion to Turbin *et al.* (1975), pointing out at page 69 (in line with Kleinhofs & Behki) that, *"This strict correlation of the two characters [wild-type pollen in two-rowed, and waxy pollen in six-rowed barley] is unexpected because they are located on different chromosomes."*

9. Hess (1978) indicates that for Turbin *et al.* (1975) working with barley, as with others' research in a number of different species in the 1970's, *"In none of these cases, however, the artificial gene transfer seems to be proven beyond any doubt."*

10. In my opinion, Turbin *et al.* (1975) do not report a method for stable plant transformation *per se*. They simply claimed to achieve a proportion of pollen cells expressing apparently modified characteristics. The authors state at page 67, *"The question to be solved is what proportion of embryonic cells may be affected by the injection of DNA into grains at a milk maturity stage? At this stage the apical meristem of the embryo comprises several cells. The degree of their competence, the probability of DNA penetration into competent cells, the fate of the injected DNA - all these questions are still open."*

11. Moreover, Turbin *et al.* (1975) state at page 67, *"So far it is not understood whether cells of one flower or a few cells of every anther of a plant underwent changes. It will become much clearer when all seeds are sown and the pollen of the progeny is examined carefully."* This is in contrast to the assertion by the Examiner at page 7 of the Office Action where the Examiner states that "Turbin *et al.* teach a method of transforming a barley plant."

12. In my opinion, Turbin *et al.* (1975) make no claim to transform a barley plant but rather at page 67 state that, “*The aim of the present work was to find out whether various DNAs have specific actions on such recessive mutant character as lack of amylose in starch grains*”. In other words, it was simply a study of gene expression.

13. Using an analogous method to Turbin *et al.* (1975), Sanford *et al.* (1984) achieved zero transformations out of 22,300 potential transformation events with this approach. Sanford *et al.* (1984) at page 556 point out that while Turbin, along with others, had proposed microinjection of foreign DNA into the egg or embryo, “. . . *no significant result has been reported*”.

14. Holl and Olson (1983) at page 171 point to the possibility of, “. . . *contamination of the DNA preparations.*” In assessing reports similar to Turbin *et al.* (1975) and at page 172 they specifically question the validity of the findings of Turbin *et al.* (1975), stating “. . . *these results too have to be challenged.*” Holl and Olsen (1983) at page 175 then go on to explain that, “*the often inconclusive and contradictory evidence of DNA transformation in plants has led to considerable resistance to accept that direct addition of DNA can produce demonstrable and heritable effects.*” They point out that by the beginning of the 1980’s, the largely unsuccessful approach of directly added DNA was being “eclipsed by” systems such as vectored transformation with *Agrobacterium*. Despite this teaching, the Henry *et al.* invention, in fact, provides a successful *directly added* DNA approach to genetic transformation, namely by microprojectile bombardment. This method is, however, completely different from that unsuccessfully explored by Turbin *et al.* (1975).

15. In a major review at the time (almost 250 citations), Scowcroft (1977) cites the paper of Turbin *et al.* (1975), but provides no discussion of its contents. I interpret this lack of discussion as a lack of acknowledgement that the results described in Turbin *et al.* (1975) are of any significance to the relevant art.

16. In summary, Turbin *et al.* (1975) report work conducted nearly 30 years ago. In the intervening period, and up to the time of the October 22, 1998 priority date of the present application, despite the development of numerous cereal transformation protocols, the Turbin method has not been utilized. The basic tenet of the approach is that if exogenous DNA is

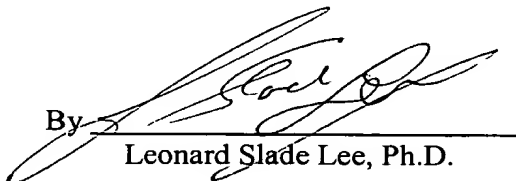
presented to a symplastic heterokaryon it will be more readily incorporated into the recipient cell due to the relatively low cell number and the absence of cell walls in the target. This is analogous to the pollen-tube pathway transformation approach. Even with the benefit of a quarter century additional research worldwide since Turbin *et al.*, (1975), Huixia Shou *et al.* (2002) at page 325 still remark that “. . . pollen-tube pathway transformation is not reproducible.”

17. Further to this, it is clear that there is no evidence that the work of Turbin *et al.* (1975) is either acclaimed or even repeatable; and the procedure has never been adopted by persons skilled in plant genetic engineering. In fact, the work of Wan and Lemaux (1994), nearly twenty years after that of Turbin *et al.* (1975), is accredited as the first efficient transformation of barley (referred to in Jacobsen *et al.* 2000).

18. The two-decade hiatus between the procedure of Turbin *et al.* (1975) and the first acknowledged barley transformation, together with the demonstrated fact that the Turbin procedure was neither efficacious nor acclaimed, supports my argument that Turbin *et al.* (1975) would not be a document that a person of ordinary skill in the art would seek to combine with Christou (1997) as of the October 22, 1998 priority date of the present application. There is simply no motivation to adopt the teaching of Turbin *et al.* (1975) given that the teachings of Turbin *et al.* have been either ignored or discredited by persons skilled in plant genetic engineering.

I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 28th Oct '03

By 
Leonard Slade Lee, Ph.D.

Enclosures: List of Cited References; Copies of Eleven (11) References

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